

Aluminium and calcium lake pigments of Lac natural dye

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This work presents the physicochemical properties of the prepared lac lakes. The study revealed the stability, cytotoxicity and use of lac lake pigment as a cosmetic colorant. SEM, XRD and DSC were used to study the characteristics of the lac lakes. The color stability of lac dye and its lakes was investigated at ambient temperature and at 45 °C, in sunlight, fluorescent light and darkness, for 3 months. Cytotoxicity on human dermal skin fibroblast cell lines was tested using MTT assay and the use of the lakes as a colorant in lip color product was studied. Different metal salts resulted in lac lakes of different shades and appearance. SEM, XRD and DSC results indicated that the morphology and structure of lac dye were changed after reacting with metal salts which support the coordination of metal ion with lac dye. The lac lakes were not cytotoxic to human dermal skin fibroblast cells (0.78-100 µg/mL). Lac lakes exhibited less alteration of color shade over acidic and basic conditions when compared with the lac dye itself. Additionally, the lake pigments displayed better color stability than lac dye and produced an orange-brown shade of lip color product. The results suggest that lac lake pigments have good potential for use as cosmetic colorant.

Keywords: Laccic acids. Natural lac dye. Lake pigment. Cosmetics.

INTRODUCTION

Lac natural dye is a red colorant obtained from the insects *Laccifera lacca* and *Kerria lacca* (Kongkachuichay, Shitangkoon, Chinwongamorn, 2002b). Lac dye is a mixture of at least five closely related laccic acids derived from 2-phenylanthraquinone. These five acids are laccic acids A, B, C, D and E (Figure 1), with a major amount of laccic acid A (71-96 %) and B (20 %) (Erwin, 2008). Lac dye was reported to possess medicinal properties for treatment of boils, burns and controlling blood flow after birth (Basu, Haq, Chaudhuri, 1996). It also exhibited strong *in vitro* antioxidant activity (Jimtaisong, Jantadee, Nakrit, 2013). With its bright and attractive red color together with its non-toxicity to human health; it has been used as material for food coloring (Divya *et al.*, 2011; Oka *et al.*, 1998; Hua, Hong, Zhongue, 2003; Hong *et al.*, 2011).

Laccic acids are water soluble dyes with an anthraquinoid type structure (Burwood *et al.*, 1965; 1976). The water soluble dye can be converted into an insoluble pigment by making it into a lake pigment. This pigment

exhibited improved staining ability and good resistance to heat, light and oxygen (Taeko, Keiko, 1980; Cosentino, Fontenele, Del Mastro, 2005). Lake pigments are produced by the precipitation of a particular dye with metal salt (Vera de Rosso, Mercadante, 2009; Anstead, 1959). Carmine is an example of a red lake pigment produced through the reaction of natural cochineal (carminic acid) and aluminium salt, and it is currently used in foods and cosmetics (Schmidt-Jacobsen, Frandsen, 2010). Carminic acid is an anthraquinone type dye with a bright red shade, and is highly resistant to light-induced-fading (Cosentino, Fontenele, Del Mastro, 2005; Schmidt-Jacobsen, Frandsen, 2009). We have previously studied the preparation and characterization of the aluminium and calcium lake pigment of lac natural dye (Wongwad *et al.*, 2012). This paper deals with the physicochemical properties of the lac lake pigments. Scanning electron microscopy (SEM), X-ray diffraction (XRD) and differential scanning calorimetry (DSC) analyses are revealed. Color stability, cytotoxicity and the application of lake pigment in color makeup were also studied.

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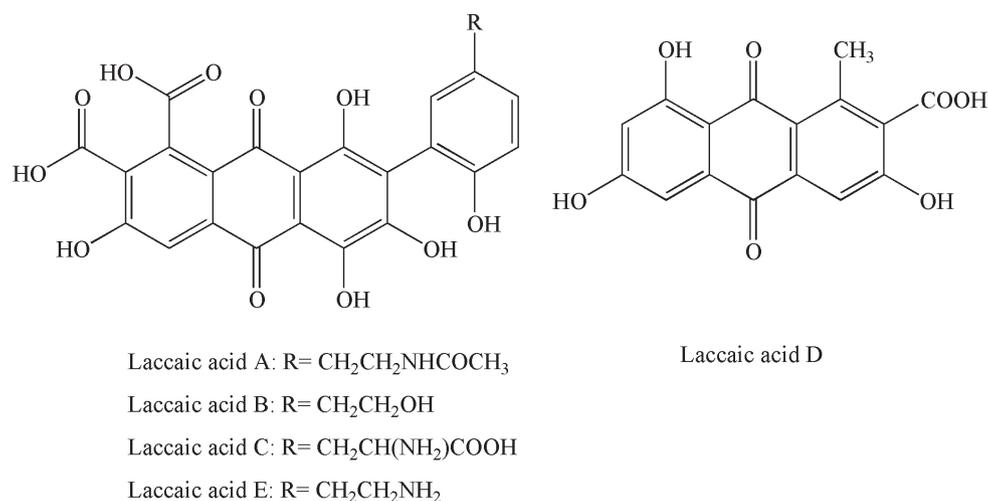


FIGURE 1 - Chemical structures of laccaic acids.

MATERIAL AND METHODS

Material

Deionized (DI) water (Milli-Q/Millipore, USA) was used as the solvent for all preparations. Sodium hydroxide (NaOH) came from UNILAB, Philippines. Calcium carbonate (CaCO₃) was obtained from Chemex, USA. Calcium acetate monohydrate (Ca(CH₃COO)₂·H₂O) originated at POCH SA, Poland. Aluminium potassium sulphate or alum (AlK(SO₄)₂·12H₂O) and aluminium chloride (AlCl₃) were procured from Ajax Fine Chem, Australia. All chemicals are of technical grade. Lac natural dye (industrial grade) was purchased from Creasia Mill Co., Ltd., Thailand. All chemicals were used as received.

Methods

Preparation of lake pigments

The lake pigments were prepared in accordance with the previous study (Wongwad *et al.*, 2012). Lac natural dye (2 g) was dissolved in 50 mL of DI water and the pH of lac dye solution was adjusted to 3.5 ± 0.1 using 1%w/v NaOH. Each metal salt solution (10 mL, 10 %w/v), i.e., aluminium potassium sulphate (alum), aluminium chloride, calcium acetate monohydrate, and calcium carbonate, was added into the reaction mixture. Reactions of aluminium and calcium salts were prepared under different conditions (Wongwad *et al.*, 2012). The aluminium lake was prepared by heating at 100 °C for 2 h, while the calcium lake was prepared at ambient temperature for 30 min. The reaction mixture was filtered using filter paper at room temperature and incubated at 45 °C overnight. In addition, a mixed-metal salt lake pigment was prepared by using aluminium potassium sulphate and calcium acetate monohydrate.

Scanning electron microscopy (SEM)

The surface morphology of lac dye and its lake pigments was investigated by scanning electron microscopy (SEM, JEOL/JSM- 5910 LV, Japan). The surfaces of the samples were coated with gold as a conductive material.

Differential scanning calorimetry (DSC)

This analysis required placing a sample in an aluminium dish before testing. The DSC measurements were performed on a Differential scanning calorimeter (METTLER TOLEDO/DSC822e, Germany) with a thermal analyzer. Samples (1.0-3.0 mg) were placed and sealed in aluminium pans, before being heated under a nitrogen flow (50 mL/min) at a scanning rate of 10°C/min from 35 to 250°C. An empty aluminium pan was used as a reference (control).

X-Ray diffraction (XRD)

Powder XRD patterns were obtained at room temperature using a XRD diffractometer (PANalytical/X'Pert Pro MPD, The Netherlands), with a Cu K α target tube, NaI detector, variable slits, a 0.050 step size, operated at a voltage of 30 kV, 15 mA current, at 2 theta (2θ)/min scanning speed, and scanning angles ranging from 0 to 80°.

MTT cytotoxicity test

The MTT assay was a modified version of conventional direct and indirect contact tests conformed to the published standard method (BS-EN30993-5 and ISO10993-5). The human dermal skin fibroblast cells were seeded in a 96-well plate at a density of 6,000 cells/well and incubated for 48 h. The samples (6.25-100 µg/mL) were added to the cells and incubated for 24 h. The test samples were removed from the cell cultures and the cells

were reincubated for a further 24 h in a fresh medium and then tested with MTT assay (Plumb, Milroy, Kaye, 1989). Next, 50 µl of MTT in PBS at 5 mg/mL was added to the medium in each well and the cells were incubated for 4 h. The medium and MTT were then aspirated from the wells, and formazan solubilized with 200 µL of DMSO and 25 µL of Sorensen's Glycine buffer, at a pH of 10.5. The optical density was read with a microplate reader (Molecular Devices) at a wavelength of 570 nm. The average of the 4 wells was used to determine the mean of each point. The experiments were carried out 3 times in order to obtain the values and standard deviation. The data were analyzed with the SoftMax Program (Molecular Devices) to determine the IC₅₀ for each toxin sample. A dose-response curve was derived from 8 concentrations in the test range using 4 wells per concentration. Toxic compounds are expressed as the concentration of each sample required to kill 50% (IC₅₀) of the cells compared to the control.

Effects of pH on color stability

5% of lac natural dye and its lake pigments were mixed with DI water and the pH of the mixture was measured. The pH value of each mixture was then adjusted in the range of 1-13 with the addition of 1% NaOH or 1% HCl. The color was visually observed and photographed.

Effects of storage on color stability

Lac natural dye and its lake pigments were prepared as a powder dispersion (30% lac dye or its lake pigments in talc) and oil dispersion (45% powder in castor oil). The stability was tested under 5 storage conditions: at ambient temperature, sunlight exposure, fluorescent exposure, darkness, and at 45 °C. The color change was measured for 3 months using a Chromameter (Minolta CR-400, Japan).

Application of lac lakes in lip color product

Lac natural dye and its lake pigments were used to adjust the color shade in lip color preparation. All waxes are melted at 75-80 °C. Oils were mixed with colorants and heated to approximately 75-80 °C. The oils and waxes were then mixed homogeneously and the mixture was poured into a container to examine its characteristics (color, smoothness, texture).

RESULTS AND DISCUSSION

Preparation of lake pigments

Lake pigments used in foods and cosmetics are usually prepared in the form of aluminium or calcium lakes because they have been approved for safety and non-

toxicity (Plumb, Milroy, Kaye, 1989). Aluminium-calcium lakes have been generated for use in foods such as yogurt fruit preparation and beverages, as well as in cosmetics (Smith, Wagner, 1991). Therefore, aluminium potassium sulphate or alum (AlK(SO₄)₂·12H₂O), aluminium chloride (AlCl₃), calcium carbonate (CaCO₃), and calcium acetate monohydrate (Ca(CH₃COO)₂·H₂O) were selected for lake preparation. The lac natural dye was used without any purification. The resulting Al-lake complexes were dark-red solids. The alum lake possessed a more brilliant, glossy and homogeneous texture than that of Al chloride. The Ca-lake complexes possessed dark-violet precipitates, and the Ca acetate lake was more glossy and homogeneous than that of Ca carbonate. It may be noted that lake pigments prepared using alum and Ca acetate were relatively high in yield, showed smooth and fine textures and are more preferable for cosmetics (Wongwad *et al.*, 2012). Thus, these salts were selected for the preparation of the mixed-metal lake complex. Treatments of the dye solution with alum and Ca acetate salt were performed by either adding the alum first, followed by adding Ca acetate, or vice versa. The results presented that when alum was added first, the Al-Ca lake, it obtained fine, glossy, dark-red precipitates. When Ca acetate was added first, the Ca-Al lake, it was less glossy, producing a darker color, Figure 2.

Characterization

Scanning electron microscopy (SEM)

Lac natural dye (1) showed fine platelet features as can be seen in Figure 3. SEM analysis of the alum lake (B) and the Al chloride lake (C) revealed that the surface appearances of both lakes are grouped in the grape fruit shape. The Ca carbonate (D) and Ca acetate lakes (E) possessed particularly rough and uneven shapes and the CaCO₃ lake seems to be bigger than that of Ca acetate. SEM analysis of the mixed-metal salts lakes, (F) and (G), showed different surface morphology. The Al-Ca lake has a relatively rounded and smaller shape, while the Ca-Al lake possessed configurations that varied in both size and shape. SEM results indicated that the complexation between lac dye and metal salts changed the lac dye features and may suggest that this occurs due to the interaction between metal salts and lac dye.

Differential scanning calorimetry (DSC)

The DSC thermograms of lac dye and its lakes are shown in Figure 4. Lac dye (1) exhibited two positions of endothermic peaks; this may be due to containing more than one major compound (Erwin, 2008). When lac dye was prepared as lake pigments by using various metal



FIGURE 2 - Precipitates of metal lake complexes.

salts, the shift in the endothermic peak minima to lower temperatures was observed in metal complexes (**B**, **C**, **D**, **E**, **F** and **G**). The DSC results may indicate that the metal ions might loosely coordinate with the OH group of lac dye (Wongsooksin *et al.*, 2008; Abdelhedi *et al.*, 2008).

X-Ray diffraction (XRD)

The XRD pattern of lac natural dye (**1**) exhibited its characteristic crystalline peaks at around 5° , 13.5° , 15.5° , 17.5° , 23.5° , 25° , and 26.5° . For Al lakes, the crystalline peaks showed at 5° , 13.5° , 21° and $\sim 27-28^\circ$ for the alum lake (**B**) and only a broad and weak peak at $27-28^\circ$ for the Al chloride lake (**C**). The XRD pattern of the Ca acetate

lake (**E**) showed a strong crystalline peak at 28° while the Ca carbonate lake (**D**) displayed a weakened peak at around $27-28^\circ$ and many new diffraction peaks at 23.5° , 30° , 36° , 39.5° , 43° , 47° , and 48° . XRD analysis was also performed on the mixed metal complexes. The Al-Ca lake (**F**), and Ca-Al lake (**G**) produced diffractograms which varied from lac dye; showing a strong crystalline peak at $\sim 27-28^\circ$ and a broad, weak peak at $41-42^\circ$ (Figure 5). The XRD patterns indicate the crystalline nature of the complexes. It is clear that the pattern of lac dye differs from its metal complexes or lake pigments, which may be attributed to the formation of a new, solid, crystalline structure. Presumably, this behavior is due to the coordination of metals with the electron-donating hydroxyl groups present in lac dye molecules (Kongkachuichay, Shitangkoon, Chinwongamorna *et al.*, 2002a; Neelakantan *et al.*, 2008).

MTT cytotoxicity test

The survival rate (%) of cells cultured with samples of various tested concentrations (compared to the control) were tabulated in Table I. The results indicated that all samples were nontoxic to human dermal fibroblast cells over the ranges of tested concentrations ($0.78-100 \mu\text{g/mL}$). Surprisingly, the lac dye and the Ca acetate lake seemed to stimulate rather than inhibit growth of human dermal fibroblast cells. It has been previously reported that lac dye (food grade, purity 95% and above) is non-toxic at the test dose of 200 mg/kg in the diets of male rats. As it shows no sign of toxicity, it has great potential use as a natural food additive (Srivastava *et al.*, 2103). The results obtained from this study are likely to be the first research into the human dermal fibroblast toxicity of lac dye and may indicate that lac dye and its lake pigments are potentially safe for topical use. However, to ensure their safety, further study on the toxicity of lac dyes on the dermal skin from humans is suggested.

Effects of pH on color shade

5% of lac dye and its various pigments were mixed with DI water and the pH of the mixture was measured. Lac dye itself has a pH of 2.99 and its lake pigments have differing pH values, i.e., alum lake, 3.16; Ca acetate lake, 5.59 and Al-Ca lake, 4.71. The pH value of each solution was then adjusted to the range of 1-13 by adding 1% NaOH or 1% HCl. The color shade of lac dye and its lake pigments are displayed in Figure 6. It can be seen that, at different pH values, lac dye possesses a higher diversity of color shade than its lake pigments. The Al-Ca lake seems to exhibit less alteration in color shade over a wide range of both acidic and basic pH values. It

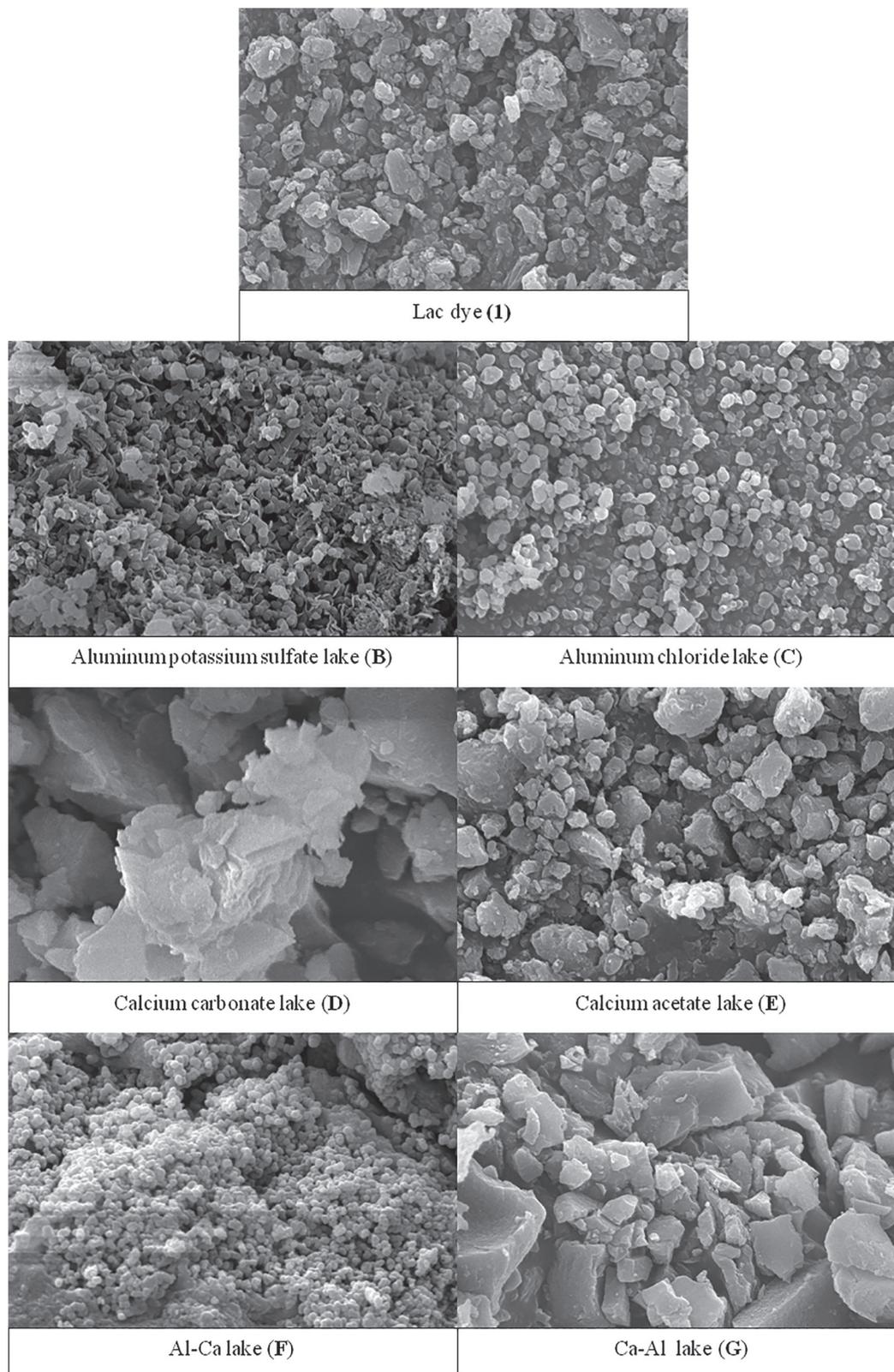


FIGURE 3 - SEM analysis of lac dye and its lake pigments.

may be concluded that lac lake pigments exhibit better stability, or greater tolerance, over extreme pHs, than the lac dye itself.

Effects of storage on color stability

The color stability of lac dye and its lake pigments was tested under 5 conditions: ambient temperature

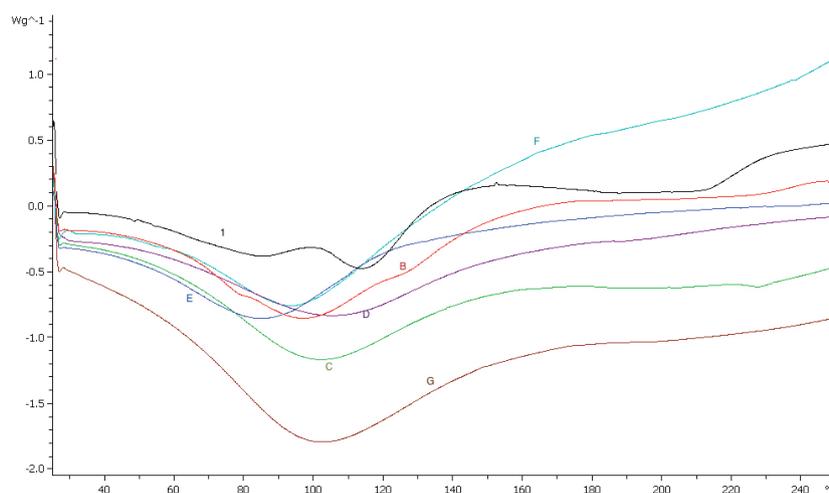


FIGURE 4 - DSC thermogram of (1) lac dye, (B) Alum lake, (C) Al chloride lake, (D) Ca carbonate lake, (E) Ca acetate lake, (F) Al-Ca lake, and (G) Ca-Al lake.

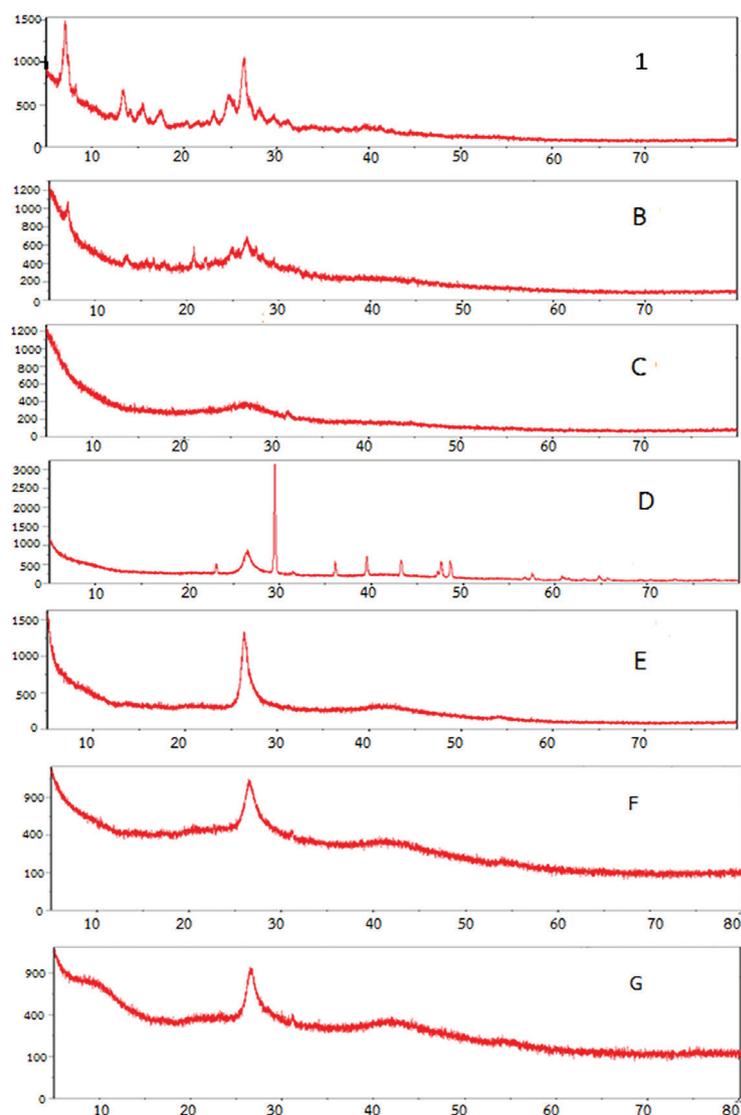
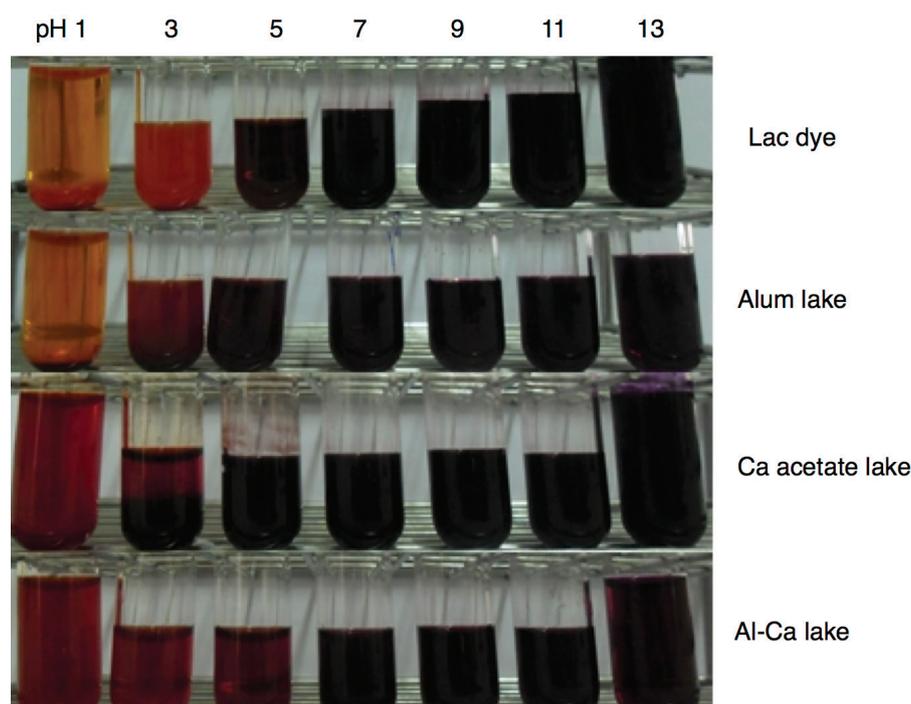


FIGURE 5 - X-ray diffractograms of (1) lac dye, (B) Alum lake, (C) Al chloride lake, (D) Ca carbonate lake, (E) Ca acetate lake, (F) Al-Ca lake, and (G) Ca-Al lake.

TABLE I - The survival rate (%) of human dermal fibroblast cells tested by MTT assay

Concentration ($\mu\text{g/mL}$)	% Survival \pm SD			
	Lac dye	Alum Lake	Ca acetate lake	Ca-Al Lake
100	147 \pm 9	98 \pm 1	116 \pm 7	95 \pm 5
50	137 \pm 2	100 \pm 5	113 \pm 8	98 \pm 4
25	126 \pm 3	93 \pm 3	102 \pm 2	94 \pm 3
12.5	109 \pm 2	99 \pm 1	109 \pm 2	95 \pm 4
6.25	97 \pm 5	96 \pm 3	93 \pm 2	96 \pm 3
IC ₅₀	> 100	> 100	> 100	> 100

**FIGURE 6** - Color shades of lac dye and its lake pigments at different pH values.

and 45 °C sunlight exposure, fluorescent exposure, and darkness. Lac dye and its pigments were tested in powder dispersions (30% lac dye or its lake pigments in talc) and oil dispersions (45% powder in castor oil). The color change was measured by a Chromameter. The total color difference (ΔE^*) between week 0 and week 12 was calculated using the equation: $\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$ (Gonnet, 2001). After 3 months, the lac dye itself showed more change in color than its lakes. The ΔE^* values of lac dye are relatively higher than those of its lake pigments. In the powder dispersions, the ΔE^* of lac dye is higher than its pigments in all conditions. The only exceptions were for those at 45 °C in which the values were relatively close to each other (Table II). In oil paste form, the change in color of lac dye is much more visible than for lac lake pigments. It should be noted that at 45 °C, lac dye in both powder and

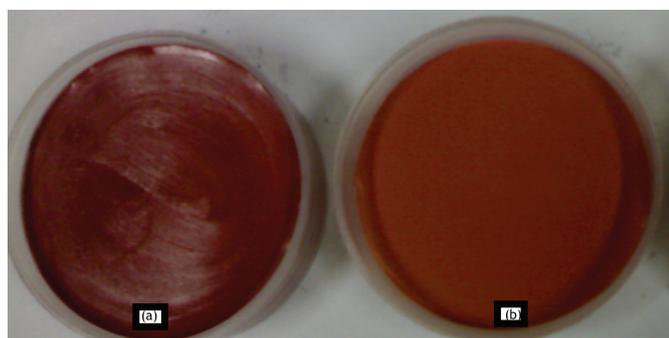
oil dispersion forms is quite stable, as the ΔE^* values are relatively less than for those of other storage conditions.

Application of lac lakes

Lac dye and lake pigment were used as the coloring agents in lip color product at 5.3%. The formulas contain 22% cosmetic waxes, 66% cosmetic oils, 2.7% red iron oxide and 4% TiO_2 . The product prepared using the lac dye produced an orange-red color while the formula containing the Al-Ca lake pigment displayed an orange-brown color (Figure 7). Both products possessed similar smooth textures but it seems that the product containing lake pigment is better in term of skin adhesion than that of lac dye. A simple test was performed to demonstrate this issue by applying the same amount of product onto a

TABLE II - The total color difference (ΔE^*) between week 0 and week 12 of lac dye and its lake pigments

Condition	ΔE^*							
	Lac dye		Alum lake		Ca acetate lake		Al-Ca lake	
	powder form	oil paste form	powder form	oil paste form	powder form	oil paste form	powder form	oil paste form
Ambient temperature	3.03	13.22	2.08	3.66	1.86	2.45	2.67	2.54
Sunlight	3.46	13.69	2.28	3.12	0.52	1.71	0.93	1.48
Fluorescent	2.96	12.40	2.80	3.97	1.45	0.30	0.90	0.99
Dark room	4.97	13.50	2.30	3.59	1.09	2.03	1.40	2.56
45 °C	1.29	7.43	0.91	3.49	2.86	2.42	1.69	0.71

**FIGURE 7** - Color shade of lip color products containing (a) Lac dye, (b) Al-Ca lake pigment.

4 cm² area of skin. The color was then wiped with cotton moistened with micelle cleanser. The color of the product with lac lake adhered to the skin better than that with lac dye (Figure 8).

CONCLUSION

The preparation of lac lake pigments using different Al and Ca salts resulted in pigments of varying physicochemical properties. SEM analysis demonstrated that the surface morphology of lac dye, which has characteristically fine particles, was changed after reacting with metal salts. The XRD and DSC studies indicated

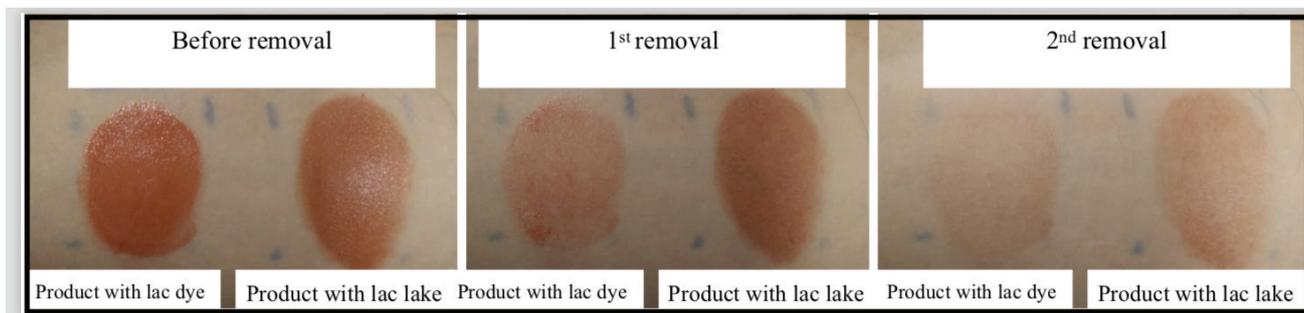
that the crystalline structure of lac dye was changed after coordinating with metal salts and a new solid compound was formed. The prepared lac lakes showed less alteration of color shade over acidic and basic pHs and also exhibited better color stability compared to lac dye. Lac dye and its lake pigments are not cytotoxic to human dermal skin fibroblast *cells in vitro*, and showed strong potential for use as cosmetic colorant.

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**FIGURE 8** - Color adhesion of the lip color products on the skin.

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